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CHIRAL STATIONARY PHASES FOR THE GAS–LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS

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CONTENTS

1. INTRODUCTION

The gas—liquid chromatographic (GLC) separation of enantiomers can be achieved by the use of chiral and achiral (conventional) stationary phases[1]. Separation on achiral phases requires the conversion of enantiomers to diastereoisomers with suitable chiral derivatization reagents prior to GLC analysis. The use of a number of chiral reagents for diastereoisomer preparation and subsequent separation on achiral stationary phases has recently been summarized[2].

Disadvantages associated with chiral derivatization reagents include the requirement of an active functional group for the formation of diastereoisomeric derivatives, differences in reaction rates of different pairs of enantiomeric compounds and difficulties in obtaining optically pure reagents[3]. In addition, the method cannot be employed if the diastereoisomeric mixture is not chemically and stereochemically stable under gas chromatographic conditions[4].

With the recent introduction of chiral stationary phases, the direct GLC resolution of enantiomers is possible. Since 1966[5,6], a variety of chiral phases have been developed and reported in the literature. To obtain maximum separation, most of these phases were wall-coated on capillary columns. This paper presents a review of progress made in this area, with particular emphasis on phase developments, theoretical concepts and applications.

2. DEVELOPMENT OF OPTICALLY ACTIVE STATIONARY PHASES*

Table I summarizes the optically active phases that have been developed and studied to date. These phases are classified into three groups, namely, peptides, diamides and ureides [strictly named carbonyl bis(amino acid esters)]. Although numerous chiral phases are included in Table 1, to the authors' knowledge, only Chirasil-Val (phase B-2 in Table 1) (Applied Science Labs., State College, PA, U.S.A.) and RSL-007 (B-3) (Alltech, Deerfield, IL, U.S.A.) are commercially available as wall-coated capillaries.

2.1. Peptide phases

Gil-Av *et al.*[5,6] first introduced the use of N-trifluoroacetyl (N-TFA) L-isoleucine lauryl ester (A-1) for the separation of N-TFA-α-amino acid esters on wall-coated capillaries. Enantiomer separation on N-TFA-α-amino acid ester phases has been theorized to involve readily reversible association between the enantiomeric compound (solute) and the asymmetric solvent (chiral phases) molecules. The formation of these transient diastereoisomeric association complexes was believed to involve hydrogen bonding between solute and solvent. Fig. 1A illustrates this interaction on a monopeptide ester phase[7]; only one hydrogen donor is present in each of the molecules. The R_5 and N-TFA ($COCF_3$) groups of the solute represent the formation of ester and amide derivatives for better solute stability and volatility. If a second hydrogen donor function is placed in a suitable position on the solvent molecule, the selectivity of the phase could be enhanced through the formation of three hydrogen bonds (Fig. 1B) in the vicinity of the asymmetric centers, thereby increasing the efficiency of the phase. This was the basis for the development of dipeptide ester phases[7].

Factors used to evaluate chiral stationary phases include separation efficiency, operating temperature range, retention time and thermal stability. In attempts to optimize these parameters, subsequent development of chiral phases involved modifications of the amino acid elements in dipeptide esters. Earlier phases[5,6] (A1) exhibited excellent resolution for highly volatile amino acids, but were generally unsuitable for less volatile species in that reported column operating temperatures were no higher than 110°C and gave long retention times. Other dipeptide ester phases[7,9–13], including some useful for the separation of the rather troublesome D,L-*tert.*-leucine enantiomers[14] (A-4), operated at similar temperature conditions with comparable resolution characteristics.

To obtain greater thermal stability, dipeptide ester phases were further developed using phenylalanine as one or both of the dipeptide constituents[3,12,13,15–19] (A-13, A-14, A-15). These phases, with lower vapor pressure, could be operated between 130 and 165°C and were capable of separating some less volatile amino acids. As a result of higher operating temperature, the retention times associated with previous dipeptide phases were reduced.

Further studies on peptide ester phase modification included tripeptides[7,8] (A-

* Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1

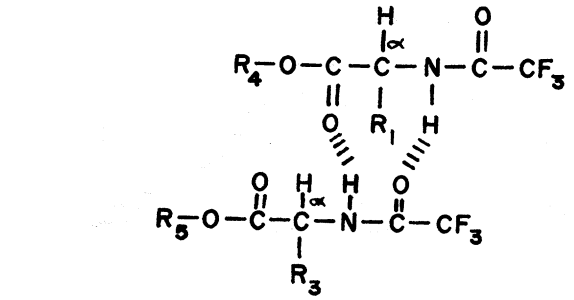
OPTICALLY ACTIVE STATIONARY PHASES

Type of phase	*Chiral stationary phase*	*Reported operating temperature (°C)*	*Application*	*Ref.*
A. Peptide phases	1 N-TFA-L-isoleucine lauryl ester	90	N-TFA-D,L-amino acid esters	5, 6
	2 N-TFA-L-alanyl-L-alanine cyclohexyl ester	110–120	N-TFA-D,L-amino acid isopropyl esters	10, 14
	3 N-TFA-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester	100–130	α- and β-amino acids, β-aminoalkanes, β-amino alcohols	9, 10, 46
	4 N-TFA-L-norvalyl-L-norvaline cyclohexyl ester	100–130	N-TFA-D,L-amino acid isopropyl esters, D- and L-*tert.*-leucine derivatives	10, 11, 14
	5 N-TFA-L-norleucyl-L-norleucine cyclohexyl ester	100–130	N-TFA-D,L-amino acid isopropyl esters	10
	6 N-TFA-L-valyl-L-valine cyclohexyl ester	90–110	N-TFA-D,L-amino acid isopropyl esters and N-TFA- and N-PFP-D,L-amino acid ethyl esters	7, 8, 12
	7 N-TFA-L-valyl-L-valine isopropyl ester	110–140	N-TFA-D,L-amino acid esters	7
	8 N-TFA-L-valyl-L-valine isopropyl ester	110–140	Same as 7	7
	9 N-TFA-L-valyl-L-leucine cyclohexyl ester	90–110	N-TFA- and N-PFP-D,L-amino acid ethyl esters and N-TFA-D,L-amino acid isopropyl esters	8, 35
	10 N-PFP-L-valyl-L-leucine cyclohexyl ester	90–110	Same as 9	8, 13, 15
	11 N-TFA-L-leucyl-L-valine cyclohexyl ester	90–110	Same as 9	8
	12 N-TFA-L-leucyl-L-leucine cyclohexyl ester	90–110	Same as 9	8
	13 N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester	130–140	N-TFA-D,L-amino acid isopropyl esters	13, 15–17

TABLE 1 *(continued)*

Type of phase	*Chiral stationary phase*	*Reported operating temperature (°C)*	*Application*	*Ref.*
	14 N-TFA-L-phenylalanyl-L-phenylalanine cyclohexyl ester	130–165	*N*-(L-α-Chloroisovaleryl)-(±)-amines and D,L-amino acid methyl esters, N-PFP-D,L-amino acid isopropyl esters	3, 12
	15 N-TFA-L-phenylalanyl-L-aspartic acid bis(cyclohexyl) ester	130–165	N-PFP-D,L-amino acid methylbutyl esters	3, 12, 18
	16 N-TFA-L-methionyl-L-methionine cyclohexyl ester	70–150	N-TFA-D,L-amino acid isopropyl esters	19
	17 N-TFA-(L-leucyl)$_2$-L-leucine cyclohexyl ester	90–110	N-TFA-D,L-amino acid isopropyl esters, N-TFA and N-PFP-D,L-amino acid ethyl esters	8
	18 N-TFA-(L-valyl)$_2$-L-valine isopropyl ester	80–140	N-TFA-D,L-amino acid *tert.*-butyl esters	7
	19 N-TFA-L-prolyl-L-proline cyclohexyl ester	110	N-TFA-D,L-proline esters	6, 44
	20 N-Caproyl-L-valyl-L-valine cyclohexyl ester	100–160	N-TFA-D,L-amino acid methyl and isopropyl esters	47
	21 N-TFA-sarcosyl-L-proline cyclohexyl ester	110	Proline	44
B. Diamide phases	1 Diamide phases derived from L-valine	90–190	N-TFA-D,L-amino acid methyl esters, N-PFP-D,L-amino acid isopropyl esters	20–23
	2 N-*tert.*-Butyl-L-valinamide/polysiloxane (Chirasil-Val)	90–190	N-PFP-D,L-amino acid isopropyl esters, N-PFP derivatives of sympathomimetic drugs, D-penicillamine, D,L-lactic acid, L-DOPA, carbohydrate and L-TPC derivatives of amphetamine and methamphetamine	2, 24–29
	3 L-Valine-*tert.*-butylamide/modified polycyanopropylmethyl silicone (RSL-007)	90–190	N-PFP-D,L-amino acid isopropyl esters, PFP-D,L-norephedrine	30
	4 N-(1*R*,3*R*)-*trans*-Chrysanthemoyl-(*R*)-1-(α-naphthyl)-ethylamine	110	Chrysanthemic acid as *tert.*-butylamide derivatives	31

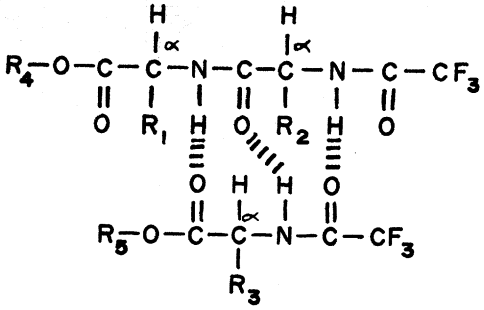
	5 N-Lauroyl-(*S*)-proline-(*S*)-1-(α-naphthyl)ethylamide	160	1-Phenyl-2-(4-tolyl)-ethylamine as N-PFP derivatives	32
	6 O-Benzyloxycarbonyl-*S*-3-phenyllactic acid-*tert.*-butylamide	62–100	O-TFA-isopropyl esters of chiral hydroxy acids, N-TFA derivatives of chiral amines	34
	7 *S*-2-Hydroxyisopentanoic acid-*S*-α-phenylethyl-amide	71–91	O-TFA-isopropyl esters of chiral hydroxy acids, N-TFA derivatives of chiral amines	33
	8 *S*-2-Hydroxyoctanoic acid *S*-α-phenylethylamide	45–100	Same as 7	33
	9 XE-60–*S*-Valine-*S*-α-phenyl-ethylamide	100–180	Carbohydrates as O-TFA-methylglycosides, N,O-bis-TFA derivatives of aliphatic α-amino alcohols	33, 34
	10 XE-60–*S*-Valine-*R*-α-phenyl-ethylamide		Same as 9	33, 34
C. Ureide phases	1 Carbonyl bis(L-valine methyl ester)	90–150	N-TFA, PFP and HFB derivatives of secondary amines	39
	2 Carbonyl bis(L-valine ethyl ester)	90–150	Same as 1	39
	3 Carbonyl bis(L-valine-*tert.*-butyl ester)	90–150	Same as 1	39
	4 Carbonyl bis(L-valine iso-propyl ester)	90–150	Secondary amine derivatives	35–37, 39
	5 Carbonyl bis(D-leucine iso-propyl ester)	60–150	Secondary amine derivatives	37–39
	6 N,N′-[2,4-(6-Ethoxy-1,3,5-triazine)diyl]-bis(L-valyl-L-valine isopropyl ester) (0A-200)	150	N-TFA derivative of α-phenylethylamine	40
	7 N,N′-[2,4-(6-Ethoxy-1,3,5-triazine)diyl]-bis(L-valyl-L-valyl-L-valine isopropyl ester) (0A-300)	180	N-TFA derivative of α-phenylethylamine and N-PFP derivative of α-phenyl-β-(*p*-tolyl)-ethylamine	40



Monopeptide ester phase (Solvent)

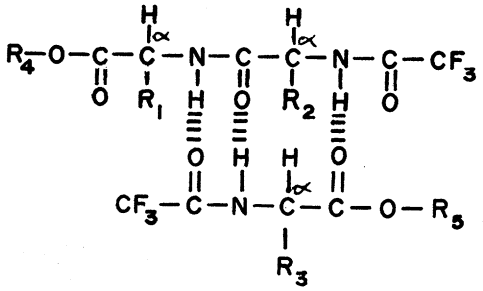
Amino acid derivative (Solute)

A



L-Dipeptide ester phase

D-Configuration of solute



L-Dipeptide ester phase

L-Configuration of solute

B

Fig. 1. (A) Monopeptide solvent–solute interaction [from B. Feibush and E. Gil-Av, *Tetrahedron*, 26 (1970) 1361, with permission]; (B) interactions of an N-TFA dipeptide ester (solvent) with D- and L-N-TFA amino acid esters (solute) [from C. H. Lochmüller and R. W. Souter, *J. Chromatogr.*, 113 (1975) 292, with permission].

17, A-18), sulfur-containing dipeptides[19] (A-16) and the substitution of an N-pentafluoropropionyl (N-PFP) for the N-TFA group[8,13,15] (A-10). Compared with corresponding dipeptide esters, tripeptide phases were found[7,8] to have similar or slightly lower resolving power and only slightly better thermal stability. Studies with sulfur-containing dipeptide phases[19] showed improved thermal stability, but failed to pro-

vide any obvious advantage in separation characteristics. N-PFP-derivatized stationary phases[8,13,15] gave shorter retention times with similar relative retentions in comparison with the corresponding N-TFA phases.

2.2. *Diamide phases*

Recognizing the essential role of the –NH–CO–C*H(R)–NH–CO group in dipeptide ester phases, the search for certain structural features of amides which might increase selectivity as well as thermal stability led to the development of an N-lauroyl-L-valyl-*tert.*-butylamide phase[20] (B-1) in 1971. Subsequent diamide phases derived from L-valine[21] exhibited greater efficiency, higher resolution factors and reduced retention times. Owing to the higher enantiomeric resolving power of these phases, packed columns were also studied[22].

To obtain better thermal stability and lower volatility, L-valine-*tert.*-butylamide was coupled to the carboxyl group of the copolymer of dimethylsiloxane and carboxyalkylmethylsiloxane[23], as shown in Fig. 2[25]. This phase was wall-coated on capillaries and is designated as Chirasil-Val (B-2). This column has been used to separate enantiomeric drugs and metabolites[25,26], amino acids of high and low volatility and some amino alcohols[27–29]. In addition, the high thermal stability of Chirasil-Val made it possible for the first time to employ a mass spectrometer coupled to a GLC system for the analysis of enantiomers[2,25].

L-Valine-*tert.*-butylamide has also been incorporated and coupled to a modified form of the well known GLC stationary phase polycyanopropylmethyl phenylmethylsilicone (OV-225)[30] (B-3). Like Chirasil-Val, this phase could be operated over a wide temperature range (60–230°C) and resolve amino acid enantiomers of both high and low volatility in one analysis. The resolution of norephedrine enantiomers was also accomplished.

Modified diamide-type phases have recently been introduced for the separation of the enantiomers of hydroxy acids, carboxylic acids and carbohydrates. Hydroxy acid enantiomers were separated on phases derived from hydroxy acids coupled to *S*-

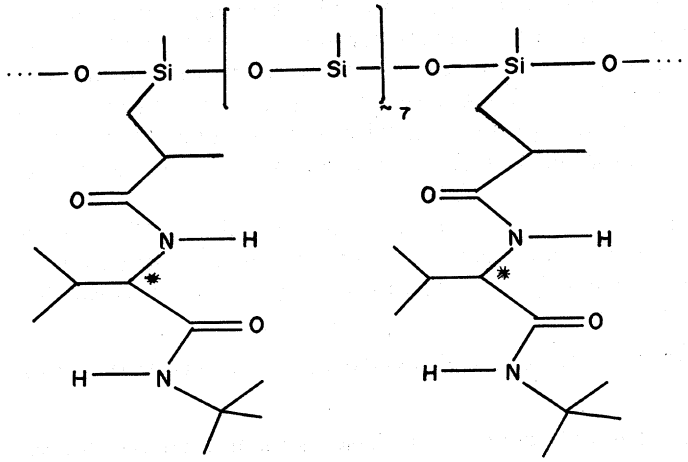


Fig. 2. Structure of the chiral stationary phase Chirasil-Val [from H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 200, with permission].

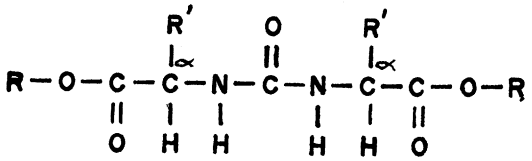


Fig. 3. General formula of ureide phases [from C. H. Lochmüller and R. W. Souter, *J. Chromatogr.*, 113 (1975) 294, with permission].

α-phenylethylamine (B-6, B-7, B-8, B-9, B-10). All four optical isomers of chrysanthemic acid were resolved by N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine[31,32] (B-4, B-5). The *R*- and *S*-isomers of 1-phenyl-2-(4-tolyl)ethylamine were resolved by N-lauroyl-(*S*)-proline-(*S*)-1-(α-naphthyl)ethylamide[31,32]. Carbohydrate enantiomers were separated on a phase prepared by coupling L-valine-*S*-α-phenylethylamide to functionalized cyanoethyl side-chains of the cyanopolysiloxane XE-60[33,34]. It was suggested[33,34] that by connecting selected chiral constituents to groups of thermally stable polysiloxanes, a variety of optically active polymers suitable for the separation of particular enantiomeric compounds may be synthesized.

2.3. Ureide phases

A third class of optically active stationary phases is the ureide type, strictly named carbonyl bis(amino acid esters). The general structure of this phase is shown in Fig. 3. Studies[35–39] indicated that enantiomeric amine derivatives could be separated by these phases in liquid[35,36], solid[36] and mesophase[38,39], depending on the operating temperature. The resolution factors for mesophases were so large that separation of solutes could also be achieved on packed columns[38].

Employing an L-valine isopropyl ester (C-4) ureide phase for the separation of enantiomeric secondary amine derivatives, it was shown[36] that larger relative retention values and much smaller capacity factors were achieved on the solid (100°C) than on the liquid (120°C) phase. The resolving power was very similar for both phases, decreasing in a similar manner with increasing temperature. D-Leucine and L-valine ureide phases (C-4, C-5) were further examined[38,39] in smectic mesophase and were found to enhance the separation of N-perfluoroacyl-2-aminoethylbenzenes. The smectic mesophase forms exhibited greater selectivity towards solutes than liquid forms and showed marked variations in relative retention with alterations in ester substituent.

A further development[40] led to modifications at the amide portion of the ureide phase. Separation of the enantiomers of arylalkylamines was carried out on N,N′-[2,4-(6-ethoxy-1,3,5-triazine)diyl] bis(L-valyl-L-valine) (C-6) and bis(L-valyl-L-valyl-L-valine) isopropyl esters (C-7).

3. SEPARATION MECHANISM AND STRUCTURAL EFFECTS OF SUBSTRATE AND CHIRAL STATIONARY PHASES

Following the introduction of peptide ester phases, structural effects on resolution factors were investigated by altering certain structural characterictics of the solute or solvent constituents at the asymmetric carbon or ester group.

It was suggested[9] that a hydrogen atom on the amide portion of the solute, although desirable, did not appear to be necessary for complex formation; the resolution of enantiomers by dipeptide ester phases required the correct arrangements of the hydrogen-bonded atoms in the solute, *e.g.*, two carbonyl groups separated by two atoms. Further studies[37] examined the hydrogen bond interactions between solutes and dipeptide esters and ureide by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectrometry. Based on these observations, it was suggested that a "single point of attachment and steric repulsion interaction" between the phase and solute was sufficient for successful resolution.

The formation of three hydrogen bonds in dipeptide ester phases might serve to bring the asymmetric centers of the two phases closer together. "When such associations occur, a particular conformation is imposed on the solute, with the acceptor and donor groups forming parts of a spiral turn, the handedness of which is determined by the configuration of the amino acids in the dipeptide"[7].

In this conformation, "the enantiomeric solutes cease to be mirror images to each other and become 'conformational' diastereoisomers". In the case of D- and L-configurations, the N-terminal region of the dipeptide phase was found to determine the elution order of enantiomers[8]. If the N-terminal region possesses the L-configuration, the phase was found to form more stable association complexes with solutes that possess the L-configuration (Fig. 1B), resulting in longer retention for these solutes[7–10,13]. It was therefore concluded[8] that the major part of the separation occurred at the amide end of the dipeptide ester.

The lengthening of R_1 and R_2 groups attached at the asymmetric carbon atoms in dipeptide ester phases (Fig. 1B) caused a general increase in ease of solute–solvent complex formation, with three and four carbon lengths competing for the highest degree of interaction[10]. A bulky ester group (R_4 in Fig. 1B), commonly a cyclohexyl group, was also important[8].

As tripeptide esters have similar separation factors, it was postulated[9] that the ester and amide ends of the molecule did not need to be in close proximity to produce a differential steric interaction.

Studies[10] on structural properties of solutes indicated that increasing the bulkiness of the group attached to the solute derivative at the asymmetric carbon atom (R_3 in Fig. 1B) produced a decrease in solute–solvent interaction. This was believed to be a simple matter of steric hindrance. Similarly, increasing the length of R_3 showed a slight inclination towards solute–solvent interactions, while a length of four carbons caused an unexpected facilitation of complex formation. However, when the side-chains of the solvent were incremented in the same manner, an increase in solute–solvent interaction was observed.

In general, it was suggested that, for best separation, peptide ester phases should be a dipeptide possessing an N-TFA group, bulky side-groups, a bulky ester group (*tert.*-butyl, isopropyl or cyclohexyl ester) and the column should be operated at the lowest feasible temperature.

While most researchers working on dipeptide ester phases explained the effect of enantiomer separation by differential hydrogen bond association complex formation, it was also suggested that molecular interaction different from hydrogen bond association, including dipole–dipole interactions and dispersion forces, must also be considered. Working with an N-TFA-L-proline–L-proline cyclohexyl ester phase[44]

(A-19), where all possible sites for hydrogen bond formation are substituted, and an N-TFA-sarcosyl-L-proline cyclohexyl ester[44] (A-21), where the number of asymmetric centers is reduced, separation was still observed for N-TFA-D,L-proline esters. Based on this evidence, together with X-ray investigations[45], it was suggested that dynamic diastereoisomeric molecular complexes originated from dipole–dipole interactions or dispersion forces are formed between molecules of solutes and the stationary phase during the chromatographic process.

The initial development of diamide and ureide phases were prompted by the feasibility of hydrogen bond formation for these two classes of compounds. The separation mechanisms for these two types of phases were assumed to be parallel to that of peptide ester phases.

The separation principle behind Chirasil-Val, a representative diamide phase[23], is very similar to that of dipeptide ester phases. Excellent thermal stability is attributed to the covalent bonding of the diamide to a very stable organosiloxane polymer network. The L-valine-*tert.*-butylamide is linked to the polysiloxane via a highly stable carboxamide group[24] (B-2). Each chiral moiety is separated from the next by approximately seven dimethylsiloxane units (Fig. 2). This arrangement avoids interaction between neighboring valine residues by hydrogen bonding, and seems to be a crucial factor for good resolution and thermal stability.

Diamide phases possessing less than seven dimethylsiloxane groups between chiral moieties were found[25] to be less thermally stable and unsuitable. Fig. 4[24] illustrates a three-dimensional view of the diastereoisomeric association complex of Chirasil-Val with O-pentafluoropropionyl-L-amino acid–cyclohexamide derivatives. The conformation resembles the pleated structure of β-keratin, permitting the formation of the maximum number of hydrogen bonds, with the methyl group from the substrate filling the space between the two isopropyl groups of the phase, and stabilized by Van der Waals forces. The space-filling isopropyl and methyl groups act as guides, keeping the diastereoisomeric association comples "on track", so to speak. For the D-enantiomer of the amino acid derivative, this stacking arrangement of hydrogen bonding groups, methyl and isopropyl groups is not possible. The result is a less stable association complex. In other words, the D-enantiomer is thrown "off track" and elutes first.

As for the separation mechanism of ureide phases, it was reported[27] that significant hydrogen bonding interaction occurred only between the N–H portion of the amide solute and the ester carbonyl of the phase. NMR evidence was interpreted[37] to indicate that only one significant portion of attachment is involved in the formation of diastereoisomeric association complexes.

The effect of changing the substituent on the donor or the acceptor strength of functional groups is also important in determining the strength of hydrogen bonding. The strength of hydrogen bonding of the ester carbonyl is dependent on the induction effect of the ester substituent. By varying the substituent, the separation factor increases from methyl to ethyl, remaining constant at isopropyl and then decreasing at *tert.*-butyl. The decrease at *tert.*-butyl was attributed to steric hindrance of the larger ester group in limiting the approach of hydrogen bonding donor solutes to the ester carbonyl[39].

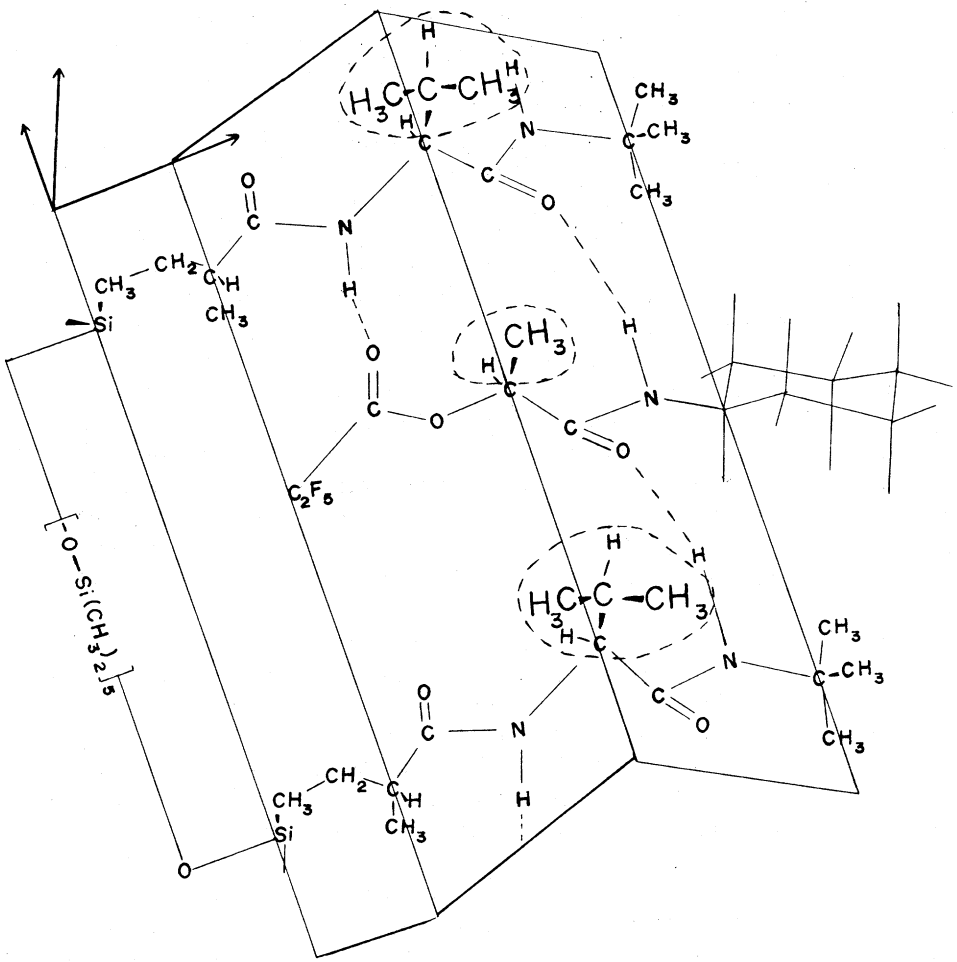


Fig. 4. Structure of the diastereoisomeric association complex between N-cyclohexyl-O-pentafluoropropionyl-L-lactamide and organosiloxane-bound N-*tert.*-butyl-L-valinamide [from H. Frank, G. J. Nicholson and E. Bayer, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 364, with permission].

4. APPLICATIONS

Since the initial development of chiral stationary phases[5,6], the separation of enantiomeric amino acid mixtures has been employed routinely to assess the separation efficiency of newly developed phases and to investigate structural effects of solvents and solutes[7–19,41–44]. Owing to limitations on the thermal stability of early chiral phases, analyses were limited to highly volatile amino acid derivatives. Further, not all amino acids could be analyzed in a single run on one phase. The introduction of N-TFA-L-norvalyl-L-norvaline cyclohexyl ester[11,14] (A-4) facilitated the separation of previously unresolved D,L-*tert.*-leucine enantiomers, and a mixture of D,L-serine and D,L-leucine enantiomers.

In general, dipeptide ester phases provided adequate separations for highly volatile amino acids. However, these columns in general have limited thermal

stability and limited column life and require lengthy analysis times for complex mixtures. The phenylalanine type dipeptide ester phases proved useful for simultaneous separations of high- and some low-volatility enantiomeric amino acid derivatives[12,13,16,17] with reduced retention times.

The early diamide phases[14] had enhanced thermal stability and were capable of achieving comparable separations to dipeptide esters with reduced retention times. However, difficulties were encountered in separating low-volatility amino acids such as proline and aspartic acid on packed columns coated with L-valine-derived diamide phases[22] (B-1). Either complete or partial separation of these two amino acids have since been achieved on capillaries coated with various dipeptide esters[3,9–12,16,46,47], but with longer retention times.

The use[23,24,30] of a diamide phase coupled to polysiloxane produced adequate separations in much shorter times. Columns were operated at up to at least 175°C without significant bleeding, allowing extended temperature programming capabilities. Polysiloxane-coupled diamides allowed the separation of both low and highly volatile amino acids in a single analysis when temperature programming was employed. Two of these phases are at present available commercially as wall-coated glass capillaries[24,30] (B-2, B-3). The improved thermal stability of these phases has expanded the analytical capability in enantiomer analysis by their suitability for coupling with mass spectrometry[2,25,26].

Chirasil-Val[23] (B-2) has been utilized to develop a reliable GLC method for the quantitative analysis of amino acids[48,49] known as enantiomer labelling. The method involves the use of the unnatural enantiomer as an internal standard. For quantitation of amino acids, a known amount of the unnatural D-amino acid is added. As D- and L-amino acids exhibit identical chemical and physical behavior, both the standard and the sample will be subjected to identical variations in recovery of sample, yields of derivatization, hydrolysis and thermal decomposition, and detector response factors[48].

This method[48,49] and a deuterium labelling technique[28] have been employed in combined gas chromatographic–mass spectrometric (GC–MS) analyses of the optical purity of natural and synthetic peptide and protein amino acid residues in biological samples. In deuterium labelling, the peptide or protein is hydrolyzed in DCl–D_2O. Amino acids from the hydrolysis are derivatized, gas chromatographed and analyzed mass spectrometrically for isotope distribution in quasi-molecular or other ions of the amino acid in the D-configuration. As the racemization of amino acids proceeds via the abstraction and re-addition of a proton from the asymmetric carbon, racemization and the incorporation of one non-exchangeable deuterium atom is a simultaneous process. The proportion of amino acids in the D-configuration originally present in the protein or biological sample could be discriminated from those arising from acid hydrolysis during sample preparation. Further application of these techniques led to an investigation on the effects of physical stress on serum amino acid levels in humans[29], and the analysis of several sympathomimetic drugs and epinephrine metabolites, lactic acid and the therapeutic agents D-penicillamine and L-DOPA[25].

A more recent contribution with potential for application in biomedical research has been the introduction of a chiral stationary phase (B-2) for the separation of non-nitrogen-containing carbohydrate enantiomers[31,50]. This phase may have

merit for future investigations into configurational analysis of mucopolysaccharides in biological systems.

Chiral stationary phases have also been applied in forensic drug chemistry. Owing to differences in pharmacological effects and degree of government regulatory measures, the development of suitable methods for the determination and differentiation of optically active drugs is needed in forensic analysis[27,51–53]. Specific procedures have been developed for the determination of amphetamine[2] and methamphetamine[26] enantiomers as N-trifluoroacetyl-L-prolyl chloride (L-TPC) derivatives by capillary column GC–MS. Advantages in combining the use of a chiral derivatization reagent with a chiral stationary phase were clearly demonstrated in these studies.

In these studies, D- and L-amphetamine mixtures were derivatized with L-TPC and then analyzed on a chiral phase (B-2) capillary GC–MS system. The total resolution of the four possible isomers (Fig. 5A) by Chirasil-Val facilitated the determination of enantiomeric impurities in commercial L-TPC and D- and L-amphetamine[2]. Under similar conditions, the four possible isomers resulting from the reaction of D- and L-methamphetamine with L-TPC (containing $<6\%$ D-TPC) could be resolved into three peaks[26] (Fig. 5B). L-Methamphetamine-L-TPC and L-methamphetamine-D-TPC were unresolved. This observation was attributed to the replacement of the active hydrogen atom attached to the nitrogen atom by a methyl group, reducing the efficiency in forming a transient diastereoisomeric association complex with the chiral phase.

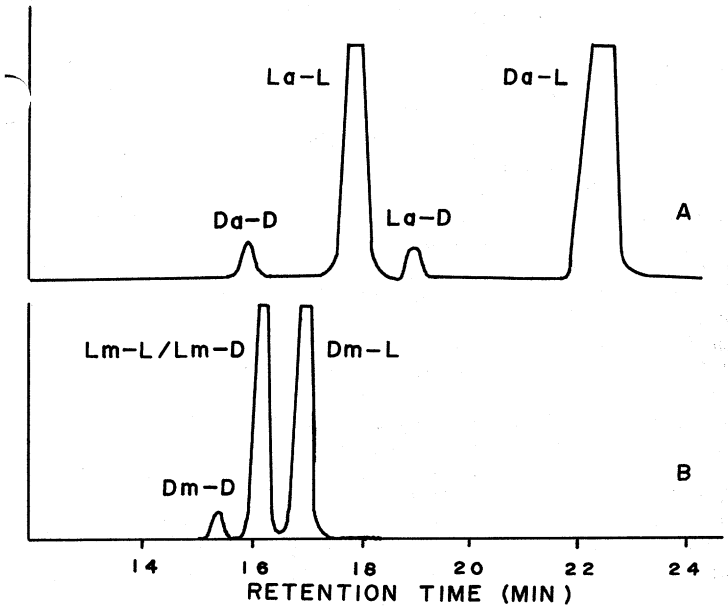


Fig. 5. Total ion chromatograms of (A) amphetamine [from J. H. Liu and W. W. Ku, *Anal. Chem.*, 53 (1981) 2182, with permission] and (B) methamphetamine [from J. H. Liu, W. W. Ku, J. T. Tsay, M. P. Fitzgerald and S. Kim, *J. Forensic Sci.*, 27 (1982) 41, with permission] as N-trifluoroacetyl-L-prolyl chloride derivatives on Chirasil-Val. Conditions for (A): 25 m × 0.30 mm I.D. glass capillary Chirasil-Val column; starting temperature, 150°C; final temperature, 180°C; programming rate, 5°C/min; carrier gas, helium at a linear velocity of 44 cm/sec. Conditions for (B): as for (A) except final temperature, 200°C. D-Amphetamine, L-amphetamine, D-methamphetamine and L-methamphetamine are designated Da, La, Dm and Lm, respectively; D-TPC and L-TPC are designated D and L, respectively.

The application of chiral phases and quantitation techniques, such as enantiomer and isotope labelling, appear to have great potential in the direct analysis of a variety of optically active compounds. More applications in these areas will undoubtedly be reported in the future.

5. SUMMARY

Three classes of chiral stationary phases used in the gas–liquid chromatographic separation of enantiomers are peptides, diamides and carbonyl bis(amino acid esters). The developments and applications of these phases, separation mechanisms and structural effects on separation efficiency are reviewed.

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